CLAIMS

1. A method of saccharifying a liquefied starch solution, which method comprises

(i) a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of

(ii) one or more high temperature membrane separation steps; and

(iii) re-circulation of the saccharification enzyme;

in which method the membrane separation steps are carried out as an integral part of the saccharification step.

2. The method according to claim 1, in which the high temperature membrane separation step is a membrane separation step accomplished at a temperature of above 63°C (preferably at a temperature in the range of from about 63 to about 80°C).

3. The method according to claim 1, wherein the saccharification step comprises of from 1 to 64 saccharification stages.

4. The method according to claim 1, wherein the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 96% DX, preferably of from about 60 to about 96% DX, more preferred of from about 80 to about 96% DX.

5. The method according to claim 1, wherein the membrane separation step comprises a microfiltration step, and/or an ultrafiltration step, and/or a nanofiltration step.

6. The method according to claim 5, wherein the membrane separation step is an ultrafiltration step.

7. The method according to claim 5, wherein the membrane separation step is a nanofiltration step.

8. The method according to claim 5, wherein the membrane separation step comprises a microfiltration step and an ultrafiltration step, preferably applied in the order specified.

9. The method according to any of claims 5-8, for the production of a dextrose preparation holding of from about 95 to about 96% DX.

10. The method according to any of claims 5-8, wherein the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 90 to about 96% DX.

11. The method according to claim 5, wherein the membrane separation step comprises a microfiltration step and a nanofiltration step, preferably applied in the order specified.

12. The method according to claim 11, wherein the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 80 to about 92% DX.

13. The method according to claim 12, for the production of a dextrose preparation holding of from about 99 to 99.9% DX.

14. The method according to claim 5, wherein the membrane separation step comprises a microfiltration step, an ultrafiltration step and a nanofiltration step, applied in the order specified.

15. The method according to claim 14, for the production of a dextrose preparation holding of from about 99 to 99.9% DX.

16. The method according to claim 1, in which the saccharification step is performed in presence of a thermostable glucoamylase enzyme (EC 3.2.1.3).

17. The method according to claim 16, in which the glucoamylase enzyme has a half-life ($T_{½}$) at 70°C of above 5-10 hours, determined in presence of 30% maltodextrin.

18. The method according to claim 16, in which the glucoamylase enzyme has a residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70°C, 0.2 AGU/ml higher than the wild-type *A. niger* glucoamylase shown in SEQ ID NO: 2.

19. The method according to claim 16, in which the glucoamylase enzyme derived from a strain of *Aspergillus*, preferably a strain of *Aspergillus niger*.

20. The method according to claim 19, in which the fungal glucoamylase is derived from a strain of *Aspergillus niger* with a substitution in position in one or more of the following positions (using the SEQ ID NO: 2 numbering): S119P, N20C, A27C, S30P+G137A.

21. The method according to claim 20, in which the *A. niger* AMG has one or the following substitution(s) (SEQ ID NO: 2 numbering): N20C+A27C+S30P+G137A; N20C+A27C; S30P; N20C+A27C+S30P; G137A; S30P+G137A.

22. The method according to claim 16, in which the glucoamylase enzyme derived from a strain of *Talaromyces*, preferably a strain of *Talaromyces emersonii*.

23. The method according to claim 1, in which is carried out in the presence of a de-branching enzyme.

24. The method according to claim 23, in which the de-branching enzyme is a pullulanase (EC 3.2.1.41) or an isoamylase (EC 3.2.1.68).

25. The method according to claim 24, in which the de-branching enzyme is a thermostable pullulanase or a thermostable isoamylase.

26. The method according to claim 25, in which the pullulanase is derived from a strain of *Pyrococcus*, in particular a strain of *Pyrococcus woesie*, or a strain of *Pyrococcus furiosus*.

27. The method according to claim 25, in which the isoamylase is derived from a strain of *Flavobacterium*, in particular *Flavobacterium odoratum*.

28. The method according to claim 1, in which the saccharification step is carried out with an added amount of a fungal α-amylase.

29. The method according to claim 28, in which the fungal α-amylase is derived from a strain of *Aspergillus*, in particular *Aspergillus niger*.

30. The method according to claim 28, in which the fungal α-amylase is derived from a strain of *Acremonuim*.

31. The method according to claim 1, wherein the membrane separation step comprises a microfiltration step and an ultrafiltration step, preferably applied in the order specified.

32. The method according to claim 31, in which the saccharification step is performed in presence of a thermostable MTSase and MTHase.

33. The method according to claim 32, in which the MTSase and MTHase is derived from a strain of *Sulfolobus*, such as *S. acidocaldarius*, especially *S. acidocaldarius* ATCC 33909.

34. The method according to claim 31, in which the liquefied starch is subjected to a thermostable CGTase.

35. The method according to claim 34, in which the CGTase is derived from a strain of the genus *Thermoanaerobacter* or *Bacillus.*

36. The method according to claim 31, in which the liquefied starch is subjected to a thermostable α-amylase and/or pullulanase and/or fungal α-amylase.

37. The method according to claim 31, in which the α-amylase is derived from a strain of the *Bacillus licheniformis* or *Bacillus stearothermophilus*.

38. The method according to claim 31, in which the pullulanase is derived from a strain of the genus *Bacillus,* such as *Bacillus acidopullulyticus* or *Bacillus deramificans*.

39. The method according to claim 31, in which the fungal α-amylase is derived from a strain of the *Aspergillus niger.*

40. The method according to claim 31, in which the liquefied starch is subjected to a β-amylase and a transglucosidase.

41. The method according to claim 40, in which the transglucosidase is derived from a strain *Aspergillus niger*

42. The method according to claim 31, for the production of a maltooligosaccharide syrup holding of from about 30 to above 80% maltose.

43. The method according to claim 31, for the production of a isomaltooligosaccharide or "Alo mixture" syrup holding of from about 10-40% isomaltose.

44. The method according to claim 31, for the production of a trehalose preparation holding of from about 75 to about 90% trehalose.

45. The method according to claim 31, for the production of a cyclodextrins preparation holding of from about 30-60% cyclodextrins.

46. A method for the production of a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of

(i) one or more high temperature membrane separation steps; and

(ii) re-circulation of the saccharification enzyme.

47. The method according to claim 46, in which the high temperature membrane separation step is a membrane separation step accomplished at a temperature of above 63°C (preferably at a temperature in the range of from about 63 to about 80°C).

48. The method according to claim 46, wherein the membrane separation step (i) is carried out as an integrated part of the saccharification step, wherein the feed stream subjected to membrane separation originates from the saccharification step, and the retentate from the membrane separation is re-circulated to the saccharification step.

49. The method according to claim 48, wherein the saccharification step comprises one or more saccharification stages, preferably of from 1 to 64 saccharification stages, more preferred of from 1 to 32 saccharification stages.

50. The method according to claim 46, wherein the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 96% DX, preferably of from about 60 to about 96% DX, more preferred of from about 80 to about 96% DX.

51. The method according to claim 46, wherein the membrane separation step comprises a microfiltration step, and/or an ultrafiltration step, and/or a nanofiltration step.

52. The method according to claim 51, wherein the membrane separation step is an ultrafiltration step.

53. The method according to claim 51, wherein the membrane separation step is a nanofiltration step.

54. The method according to claim 51, wherein the membrane separation step comprises a microfiltration step and an ultrafiltration step, preferably applied in the order specified.

55. The method according to claim 46, for the production of a dextrose preparation holding of from about 95 to about 96% DX.

56. The method according to claim 46, wherein the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 90 to about 96% DX.

57. The method according to claim 46, wherein the membrane separation step comprises a microfiltration step and a nanofiltration step, preferably applied in the order specified.

58. The method according to claim 57, wherein the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 80 to about 92% DX.

59. The method according to claim 46, for the production of a dextrose preparation holding of from about 99 to 99.9% DX.

60. The method according to claim 46, wherein the membrane separation step comprises a microfiltration step, an ultrafiltration step and a nanofiltration step, applied in the order specified.

61. The method according to claim 60, for the production of a dextrose preparation holding of from about 99 to 99.9% DX.

62. The method according to claim 46, in which the saccharification step is performed in presence of a thermostable glucoamylase enzyme (EC 3.2.1.3).

63. The method according to claim 62, in which the glucoamylase enzyme has a half-life ($T_{½}$) at 70°C of above 5-10 hours, determined in presence of 30% maltodextrin.

64. The method according to claim 63, in which the glucoamylase enzyme has a residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70°C, 0.2 AGU/ml higher than the wild-type *A. niger* glucoamylase shown in SEQ ID NO: 2.

65. The method according to claim 62, in which the glucoamylase enzyme derived from a strain of *Aspergillus*, preferably a strain of *Aspergillus niger*.

66. The method according to claim 65, in which the fungal glucoamylase is derived from a strain of *Aspergillus niger* with a substitution in position in one or more of the following positions (using the numbering of SEQ ID NO: 2): S119P, N20C, A27C, S30P, G137A.

67. The method according to claim 66, in which the *A. niger* AMG has the following substitution(s) (SEQ ID NO: 2 numbering): N20C+A27C+S30P+G137A; N20C+A27C; S30P; N20C+A27C+S30P; G137A; S30P; G137A

68. The method according to claim 62, in which the glucoamylase enzyme derived from a strain of *Talaromyces*, preferably a strain of *Talaromyces emersonii*.

69. The method according to claim 60, in which is carried out in the presence of a de-branching enzyme.

70. The method according to claim 69, in which the de-branching enzyme is a pullulanase (EC 3.2.1.41) or an isoamylase (EC 3.2.1.68).

71. The method according to claim 69, in which the de-branching enzyme is a thermostable pullulanase or a thermostable isoamylase.

72. The method according to claim 70, in which the pullulanase is derived from a strain of *Pyrococcus*, in particular a strain of *Pyrococcus woesie*, or a strain of *Pyrococcus furiosus*.

73. The method according to claim 70, in which the isoamylase is derived from a strain of *Flavobacterium*, in particular *Flavobacterium odoratum*.

74. The method according to claim 46, in which is carried out with an added amount of a fungal α-amylase.

75. The method according to claim 74, in which the fungal α-amylase is derived from a strain of *Aspergillus*, in particular *Aspergillus niger*.

76. The method according to claim 74, in which the fungal α-amylase is derived from a strain of *Acremonium*.

77. The method according to claim 46, wherein the membrane separation step comprises a microfiltration step and an ultrafiltration step, preferably applied in the order specified.

78. The method according to claim 77, in which the saccharification step is performed in presence of a thermostable MTSase and MTHase.

79. The method according to claim 78, in which the MTSase and MTHase is derived from a strain of *Sulfolobus*, such as *S. acidocaldarius*, especially *S. acidocaldarius* ATCC 33909.

80. The method according to claim 77, in which the liquefied starch is subjected to a thermostable CGTase.

81. The method according to claim 80, in which the CGTase is derived from a strain of the genus *Thermoanaerobacter* or *Bacillus*.

82. The method according to claim 77, in which the liquefied starch is subjected to a thermostable bacterial α-amylase and/or pullulanase and/or fungal α-amylase.

83. The method according to claim 82, in which the α-amylase is derived from a strain of the *Bacillus licheniformis* or *Bacillus stearothermophilus*.

84. The method according to claim 82, in which the pullulanase is derived from a strain of the genus *Bacillus*, such as *Bacillus acidopullulyticus* or *Bacillus deramificans*.

85. The method according to claim 82, in which the fungal α-amylase is derived from a strain of the *Aspergillus niger*.

86. The method according to claim 77, in which the liquefied starch is subjected to a β-amylase and a transglucosidase.

87. The method according to claim 86, in which the transglucosidase is derived from a strain *Aspergillus niger*

88. The method according to claim 87, for the production of a maltooligosaccharide syrup holding of from about 30 to above 80% maltose.

89. The method according to claim 77, for the production of a isomaltooligosaccharide or "Alo mixture" syrup holding of from about 10-40% isomaltose.

90. The method according to claim 77, for the production of a trehalose preparation holding of from about 75 to about 90% trehalose.

91. The method according to claim 77, for the production of a cyclodextrins preparation holding of from about 30-60% cyclodextrins.